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FUNGI: AN IDEAL BIOTRANSFORMATION MODEL FOR MIMICKING MAMMALIAN DRUG METABOLISM

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ABSTRACT

The concept of microbial model of mammalian metabolism was introduced for the first time by Smith and Rosazza in 1970. Since then different experiments have been performed to illustrate mammalian metabolism using microbes. The assumption is based on the fact that fungi are eukaryotes and have functionally similar enzyme system as that of mammals. This assumption was experimentally proved by studying the enzyme systems of both fungi and mammals belonging to Phase I and II (P450). The aim of this study was to review the work conducted over 40 years to show the similarity between microbial system and mammalian system.

KEYWORDS: Fungi, Microbial Model, P450 Enzyme System, Drug Metabolism

INTRODUCTION

Biotransformation is defined as structural modification of chemical molecules using biological system (Komal et al; 2012) Drugs are substances that bring about the changes in biological functions through their action. Drugs consumed by human being are lipophilic, non-polar in nature, so that they can easily penetrate through lipid bilayer and reach their target site.Since these drugs are hydrophobic in nature they are not readily excreted from the body and they can get accumulated in bilayer causing toxicity. There are few cases where same enzyme can convert functional metabolite into toxic compound that has reactivity towards other component of cell. Therefore enzyme system present in liver (cytochrome p450) converts them into hydrophilic, polar, non-toxic form and excretes them. Drug biotransformation is carried out by combination of phase I and phase II reaction (Pius et al; 2012).

Phase I: In phase one reaction drugs undergo oxidation, reduction and hydrolysis to which convert them into an intermediate: a) active drug into inactive drug, b) prodrug into active drug, c) sometimes it forms compound which retains same property of parent drug. During phase one some drug become water soluble and eliminates out but some drug does not acquire enough water solubility hence undergo phase II reaction (Jeanne Louise Schonborn and Carl Gwinnutt 2010) [Figure 1].

Phase II: Phase II reaction also called as conjugate reaction convert the intermediate into nontoxic compound by attaching molecules present in the body to reactive site of phase I product and as a result its solubility increase and it is then eliminated out of body (Jeanne Louise Schonborn and Carl Gwinnutt 2010) [Figure 1].

Up to 60% of administered dosages of drugs are either excreted as original compound or in the form of metabolites which enters the water cycle. Wastewater treatment has been found to be adequate as a result there is an

increased pressure of such unmetabolised compound in ground water, surface water and even drinking water causing hazardous effect on human life and presenting risk to the aquatic life (Rodriguez et al; 2003: Petrovic et al; 1904). Sometime these compounds are in active form and although in low concentration their continuous release has potential negative impact on environment. Degradation of such compound is a challenge because of their structure and low bioavailability (Esplugas et al; 2007; Duran et al; 2000) Conventional method of oxidation to remove such compounds fails as they have variable degradation yield, therefore an alternative approach of using fungi which has an enzyme system for detoxifying and degrading toxic end-product of drug are exploited for bioremediation. (Esplugas et al; 2007; Duran et al; 2000)

The phrase "microbial model of mammalian metabolism" was coined for first time by Smith and Rosazza (1974) to describe the system for conducting microbial hydroxylation of aromatic substrates (Osorio et al; 2008). They observed similarity between mammalian metabolite and the metabolite obtained from microbes. Ferris then conducted metabolism of various drugs and showed similarity in microbial and mammalian system (Abourashed et al; 1999).Hence on the basis of fact from experimental value Smith and Rosazza assumed that fungi and mammals might be having similar enzyme system to carry out metabolism whose outcome of metabolism was expected to be similar. The advantage of the microbial model has to predict potential routes of mammalian metabolism in the early phase of drug development. By this approach metabolites were isolated and identified, to investigate its pharmacokinetics, pharmacological and toxicological properties. This study was also used to understand degradation of drugs present in the waste water (Pius et al; 2012).

Therefore, microorganism mimicking mammalian metabolism and performing novel biotransformation and having enzymes that act on one or number of drug indicates that microbial system represents an attractive alteration to the use of mammalian system or synthesis of metabolites.

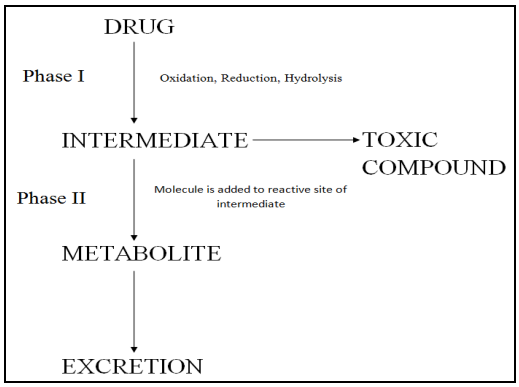


Figure 1: Drug Metabolism Pathway

DIFFERENT STRATEGIES FOR MAMMALIAN AND MICROBIAL MODEL

Working Approaches (Abourashed et al; 1999)

- **Retrospective Approach**: In such approach microbial metabolism is studied after the mammalian metabolism has conducted and their metabolites are characterized.
- **Prospective Approach:** In this approach studies are first performed on microbial system that can serve as standard forgetting information on metabolism in mammalian system. Advantage of using such approach is obtaining of higher amount of microbial metabolites that can be studied further.

- **Parallel Approach**: In this approach both microbial and mammalian metabolism are studied together and their results are then compared for further studies.

FUNGI AS MICROBIAL MODEL

The fungal kingdom consists of a wide variety of eukaryotic microorganism including yeast, mold, and basidiomycetes. Fungi inhabit a broad range of environments and play fundamental roles in nutrient cycling by decomposing organic matter. Many filamentous fungi are capable of converting environmental pollutant. They can mimic the mammalian metabolism through their p450 intra cellular enzyme system.

Cytochrome p450 are heme containing monooxgenases encoded by large super family of genes. Cytochrome p450 is key enzyme required for production of metabolite and also help fungi in adapting to different environment by detoxifying harmful xenobiotic (e.g., drugs, PAH) and many other biochemical conversions takes place by cytochrome p450 enzyme. Although cytochrome P450 enzymes carry out a wide range of biocatalytic conversions, the general reaction equation for all these reactions is summarized as:

RH + NAD(P)H + H+ + O2

= ROH + NAD(P)+ + H2O.(1)

Electrons are needed for insertion of oxygen into substrate(R), which are donated by separate electron donating group. In prokaryotes and mitochondria the electron donating system is two-protein system (adrenodoxin and adrenodoxin reductase) while a single protein (cytochrome P450 reductase, CPR2) is found in fungal system.

During the degradation of xenobiotics metabolic pattern was observed for fungi and mammal and close resembles was observed between two systems, but satisfactory formal evidence of p450 causing degradation was little. To proof this there was need for purification of the enzyme but to purify fungal p450 was extremely difficult. Hence indirect experiments (e.g. cytochrome p450 inhibitor) were performed in order to show the role of cytochrome p450 enzyme (Hans et al; 1998)

A large super family of heme containing mono oxygenase is popularly known as cytochrome p450. Numerous molecular variations among the p450 species has led to their evolution. Thus, during the course of evolution which produce a vast diversity of p450 (Hirofumi et al; 2012).

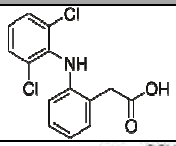
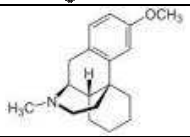
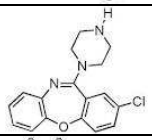
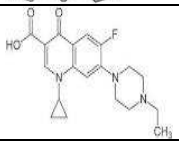
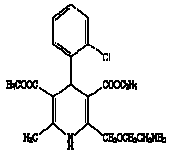
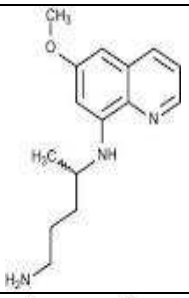
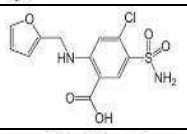
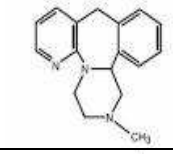
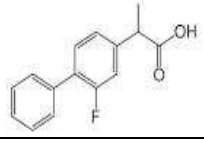
Fungi p450 Attract Much Attention in Industry Because

- Use of fungi allows reduction in the number of tedious steps unlike conventional chemical synthesis.
- A single product without byproduct formation reduces cost of purification step.
- It minimizes the use of laboratory animals.
- Diversity of metabolite production.
- Higher yield of pure metabolite obtained (Abourashed et al; 1999)

Fungi play a pivotal and promising role in drug biotransformation. They possess metabolic plasticity therefore they are called the most “inventive biological chemists” (Saxena 2009) Today metabolites are produced mostly by chemical synthesis although production by biological processes is efficient with optimum grade results and with least

waste management issues. A significant problem with the biological approach is the effective half-life of the biocatalyst which can be overcome by immobilization (Jessica 2013).

Table 1: Microbial Transformation Using Fungi

	Drug	Chemical Structure	Microorganisms	Microbial Metabolites	Human Metabolites	Mode of Action
1	Diclofenac		*Epicoccumnigrum IMI354292*	4-hydroxy diclofenac and 5 – hydroxydiclofenac	4-hydroxy diclofenac and 5-hydroxy diclofenac	Anti inflammatory
2	Dextro-methorphan		*Cunninghamella Blakesleeana AS 3.153*	Dextomethorphan, 3 hydroxymorphinan, 3-methoxy morphinan	Dextomethorphan, 3-hydroxymorphinan, 3- methoxymorphinan	Antitissuve
3	Amoxapine		*Cunninghamella elegans*	N-formyl 7-hydroxyamoxapine	7-hydroxyamoxapine	Antidepressant
4	Enrofloxacin		*Mucorramannianus*	EnrofloxacinN-oxide, N-acetylciprofloxacin, desethylene-enrofloxacin		Antibacterial
5	Verapamil		*Cunninghamella blakesleeana*	N dealkylation, O demethylation, and sulfate conjugation.	N demethylation and N dealkylation	Antimalarial
6	Primaquine		*Candida tropicalis*	8-(4-acetamido-1-methylbutylamino)-6-methoxyquinoline (II) and 8-(3-carboxyl-1-methylpropylamino)-6-methoxyquinoline (III).	7-Hydoxyamoxapine and 8-Hydroxamoxapine	Antimalarial
7	Furosemide		*Cunninghamella elegans*	Beta glucoside	areneoxide,glucorinide ,N-dealkylation product	Antihypertensive
8	mirtazapine		*Cunninghamella elegans*	8-hydroxy mirtazapine,N-desmethyl-8-hydroxy mirtazapine,N-desthymirtazapine	8 hydroxy mirtazapine, N-desmethyl-8-hydroxy mirtazapine, N-desmethyl mirtazapine, 13 hydroxy mirtazapine	Antibiotics
9	flurbiprofen		*Cunninghamella elegans*	4-hydroxy flurbiprofen	4 hydroxyflubiprofen	Antidepressant

FUNGAL BIOTRANSFORMATION PATHWAYS FOR REPRESENTATIVE DRUGS FROM FOUR GROUPS

Diclofenac

Diclofenac is clinically used as anti-inflammatory drug and has shown to be effective in treating a variety of acute and chronic pain and inflammatory conditions. As with all NSAIDs, diclofenac exerts its action via inhibition of prostaglandin synthesis by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Diclofenac metabolize in the liver by p450 (CYP) isoenzyme CYP2C9, resulted in formation of major oxidative metabolite 4'-OH-DCF and minor product 5'-OH-DCF (Takeno S and Sakai 1991: Stierlin 1979:). The generation of mammalian metabolite of diclofenac was done using white rot fungi *P.sordida* and metabolites obtained were 4-hydroxyas major metabolite, 5-hydroxy and 4-5-dihydroxy diclofenac as minor metabolites. Outcome of results of microbial system paralleled the result of mammalian system. Results were analyzed using TLC, HPLC, mass spectroscope and gas spectroscopy (Shen et al; 1999).

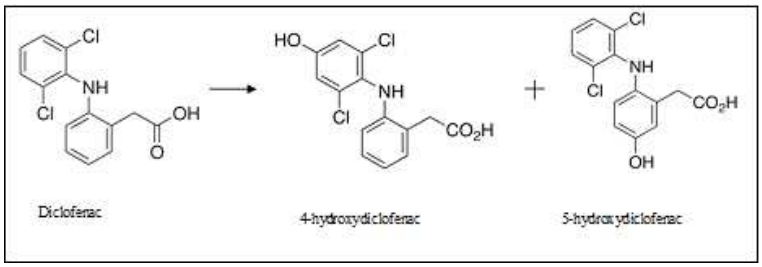


Figure 2

Mammalian Phase I metabolism of diclofenac

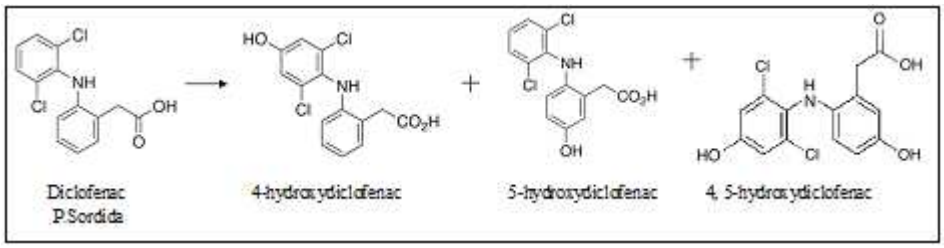


Figure 3: Metabolites of Diclofenac

Naproxen

Naproxen [(S)-6-methoxy-2 naphthalene acetic acid] is a drug and is commonly used for relief of a wide variety of pain, fever, inflammations, and stiffness.

Degradation

Naproxen was transformed by *Cunninghamella* species (*Cunninghamella blakesleana* and *Cunninghamella echinulata)* into two metabolite namely; desmethylnaproxen and desmethylnaproxen-6-O-sulfate. Both these metabolites were the known mammalian metabolites. Desmethylnaproxen is the phase I metabolite and desmethylnaproxen 6-O-sulfate, is phase II metabolite.

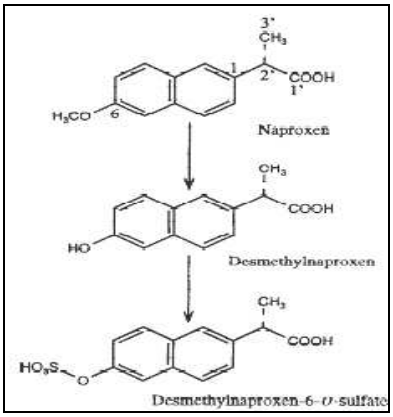


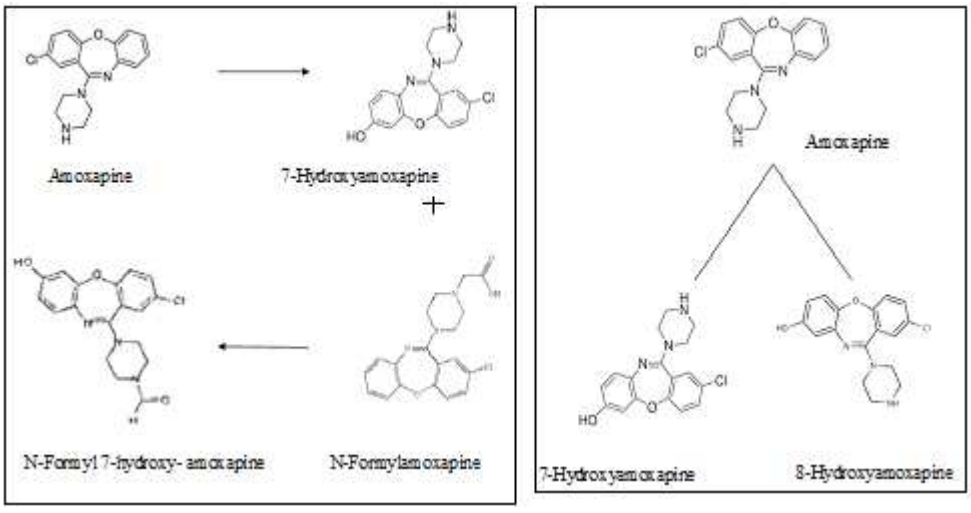
Figure 4: Metabolites of Naproxen (Zhong et al; 2003)

No conjugated metabolites were observed in the research although naproxen is mainly excreted as conjugates of naproxen and desmethylnaproxen in animals and humans.

Similarity in both the systems-Phase I metabolite (Desmethylnaproxen) was the only metabolite of naproxen in both microbial models and mammals (Hata et al; 2000)

Amoxapine

Amoxapine is a tricyclic antidepressant drug belonging to the dibenzoxazepine class and is the N-demethylated derivative of the neuroleptic compound loxapine (Zhong et al; 2007: Cohen et al; 1982). The generation of metabolites of amoxapine utilizing *Cunninghamellaelegans* was attempted to demonstrate the ability of this microbial model to mimic the mammalian system. Three metabolites were isolated. 7-Hydroxyamoxapine was the common metabolite formed by hydroxylation at C-7 position, which was found to be the major enzymatic attack. The second metabolite, N-Formylamoxapine was formed by addition of formyl group to the piperazine ring. N-Formyl 7-hydroxyamoxapine is the result of combined modifications at both the above locations. This was the third metabolite formed (Hue et al; 1998). In mammals, amoxapine undergoes hepatic biotransformation to yield two major metabolites: 7-Hydroxyamoxapine and 8-Hydroxyamoxapine (Johanno et al; 2000: Calvo et al; 1985)



In Celegan **In Mammals**

Figure 5: Metabolites of Amoxapine

Dextromethorphan

Dextromethorphan, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, is a well known antitussive drug (Takeuchi et al; 1993). In mammalian system CYP3A catalyses the N-demethylation of dextromethorphan and dextrorphan to yield 3-methoxymorphinan and 3-hydroxymorphinan respectively (Bem et al; 1992). CYP2D6 is involved in O-demethylation of 3-methoxymorphinan to 3-hydroxymorphinan. Finally, glucuronidation of dextrorphan and 3-hydroxymorphinan is carried out to give dextrorphanglucuronide and 3-hydroxymorphinan glucuronide which were detected in human urine [Figure 5]. In microbial metabolism studies using the fungi Cunninghamella blakesleeana AS 3.153, it was reported that no conjugates of dextromethorphan or dextrorphan were produced (Lutz et al; 2004). [Figure 5]

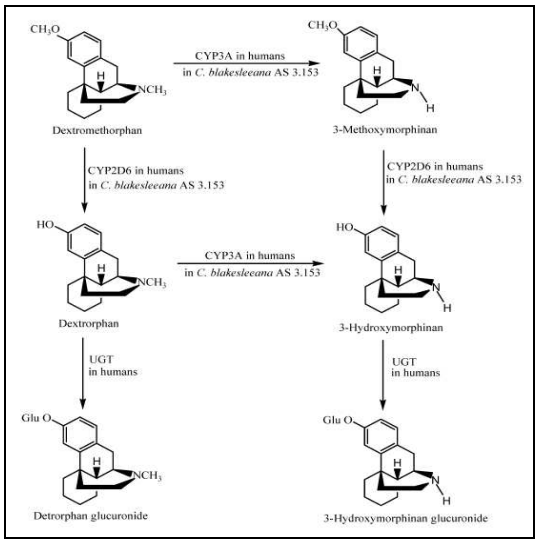


Figure 6: Proposed Metabolic Pathway of Dextromethorphan (Lutz et al; 2004)

Significance of Microbial Biotransformation Mimicking Mammalian System

Oral administration of drugs leads to deposition of poorly excreted drugs which in case increases the load on liver causing hepatotoxicity. Whereas, when microorganisms are involved in drug metabolism, directly the active principle can be obtained, which is non-toxic to the body. Due to this reason microorganisms are also used in detoxification of wastewater. In this case, the chance of formation of toxic intermediate compounds is also reduced.

CONCLUSIONS

The concept of microbial model of mammalian system is continuously being used and it has proved to be beneficial and has shown its utility in carrying out in vivo studies of drugs, reason is due to its low cost, relative simplicity and abundance of metabolite production. Fungi as eukaryotes are best suited for such application due to the similarity of cytochrome p450 enzyme system to those of the mammalian liver microsomal enzymes.

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